A review of the fusion of trigeminal and facial ganglia during larval development of some neobatrachian anurans

Marissa FABREZI & Teresa CHALABE

Museo de Ciencias Naturales,
Universidad Nacional de Salta, Mendoza 2, 4400 Salta, Argentina

The intracranial relations of the trigeminal (V) and facial (VII) nerves have been examined in larval sequences of *Ceratophrys cranwelli*, *Dermatonotus muelleri*, *Hyla pulchella andina*, *Lepidobatrachus llanensis*, *Phyllomedusa sauvagii*, *Physalaemus biligonigerus* and *Scinax fuscovaria*. Whole mounts stained for peripheral nerves and transverse histologic sections were prepared for this purpose. *H. pulchella andina*, *P. sauvagii*, *S. fuscovaria* and *P. biligonigerus* have the trigeminal and facial ganglia fused at similar stages of larval development. In later larval stages this fusion progresses to the roots of these nerves. In early stages of development of *D. muelleri* the trigeminal and facial roots are fused; in later larval stages this fusion occurs at the ganglia. *C. cranwelli* and *L. llanensis* have the trigeminal and facial nerves separated throughout their larval development. In *C. cranwelli* fusion of the ganglia takes place before metamorphosis, whereas the separation continues in *L. llanensis* until metamorphosis ends.

Bibliothèque Centrale Muséum

INTRODUCTION

3 3001 00050383 8

The importance of larval characters in anuran classification has been stressed by ORTON (1957), STARRETT (1973) and SOKOL (1975). Some of these characters have been incorporated into phylogenetic analyses of the taxon in order to define monophyletic groups (DUELLMAN & TRUEB, 1985; FORD & CANNATELLA, 1993). Among such characters one refers to the relation between the trigeminal (V) and facial (VII) nerves.

In the majority of anurans, as distinct from other vertebrates, the ganglia of the trigeminal and facial nerves are fused in a single prootic ganglion, and all rami of both

nerves emerge from the skull through a single prootic foramen, which lies immediately anterior to the otic capsule (GOODRICH, 1930; DE BEER, 1937). The fusion of these ganglia was proposed by SOKOL (1975) as a derived condition for a group of anurans and their larvae. Anurans with Type III larvae (Discoglossoidei) have separated trigeminal and facial nerves, with their rami transversing the skull through two foramina separated by the prefacial commissure. Anurans with Type IV, II and I larvae (Ranoidei) have a prootic ganglion and a single cranial foramen for exit of the trigeminal and facial rami. Different degrees of fusion of the nerve roots among the Ranoidei tadpoles were mentioned by SOKOL (1975, 1977, 1981).

The Ranoidei proposed by SOKOL (1975) were later renamed Pipanura by FORD & CANNATELLA (1993). Fusion of trigeminal and facial ganglia is included among their synapomorphies, although the information related to this character among the taxa involved is scant.

The existing information on trigeminal and facial nerves in anurans is summarized in tab. 1. This calls attention to: (1) the limited number of species in which the character has been investigated; (2) the lack of uniformity in the information, which makes comparison difficult, because different stages of ontogeny were studied.

Because data in the literature exist for only six genera, we decided to review this character in neobatrachian anurans. For this purpose, we investigated premetamorphic larval sequences of species belonging to three major anuran groups which are not closely related.

Ceratophrys cranwelli, *Lepidobatrachus llanensis* and *Physalaemus biligonigerus* are included in the "Leptodactylidae", considered a paraphyletic group (FORD & CANNATELLA, 1993). *C. cranwelli* and *L. llanensis* are in a subfamily different from that of *P. biligonigerus* (DUELLMAN & TRUEB, 1985).

Hyla pulchella andina, *Phyllomedusa sauvagii* and *Scinax fuscovaria* are members of the Hylidae, a taxon defined by a single synapomorphy (FORD & CANNATELLA, 1993). Both *H. p. andina* and *S. fuscovaria* are grouped in a subfamily different from that of *P. sauvagii* (DUELLMAN & TRUEB, 1985).

Dermatonotus muelleri is included in the Microhylidae, whose monophyly has been supported by some synapomorphies (FORD & CANNATELLA, 1993).

We analyzed trigeminal and facial nerves relation during development in these species and reviewed the available literature on this subject in order to evaluate an important character that has been considered in recent anuran phylogenies.

MATERIAL AND METHODS

The larval specimens were examined at different stages up to the beginning of metamorphosis, in accordance with GOSNER's (1960) table. The larval stages analyzed are specified for each species because representative specimens of the complete sequence were not available. The skulls of larvae and adult specimens were also analyzed in order to

Tab. 1. - Condensed information from literature in which there are observations of trigeminal and facial ganglia and roots, and prefacial commissure in anurans.

Family (LAURENT, 1986)	Genus	Author	Specimens analyzed	V and VII ganglia	V and VII roots	Prefacial commissure
Leiopelmatidae	Ascaphus	PUSEY, 1938	Ontogeny	Separated	Separated	Present
		VAN EEDEN, 1951	Ontogeny	Separated	Separated	Present
	Leiopelma	PUSEY, 1938	Ontogeny	Separated	Separated	Present
		VAN EEDEN, 1951	Ontogeny	Separated	Separated	Present
		STEPHENSON, 1951	Ontogeny	Separated	Separated	Present
Discoglossidae	Bombina	PUSEY, 1938	Adult	Separated	Separated	Present
		VAN EEDEN, 1951	Adult	Separated	Separated	Present
		SOKOL, 1975	Larval stage	Separated	Separated	Present
	Alytes	DE BEER, 1937	Larval stage	Separated	Separated	Present
		PUSEY, 1938	Adult	Contiguous	Separated	Present
		VAN EEDEN, 1951	Adult	Contiguous	Separated	Present
	Discoglossus	PUSEY, 1943	Adult	Fused	Separated	Present
		VAN EEDEN, 1951	Adult	Fused	Separated	Present
		SCHLOSSER & ROTH, 1995	Larval stage	Separated	Separated	?
Pelobatidae	Pelobates	PLASOTA, 1974	Ontogeny	Fused	?	Absent
		ROČEK, 1980	Ontogeny	Fused	?	Absent
		SOKOL, 1975	Larval stage	Fused	Separated	Absent
	Scaphiopus	SOKOL, 1975	Larval stage	Fused	Fused	Absent
Pelodytidae	Pelodytes	SOKOL, 1981	Larval stage	Fused	?	Absent
Rhinophrynidae	Rhinophrynus	SOKOL, 1975	Larval stage	Fused	?	Absent
Pipidae	Xenopus	SOKOL, 1977	Larval stage	Fused	?	Absent
	Pipa	SOKOL, 1977	Larval stage	Fused	?	Absent
	Hymenochirus	SOKOL, 1977	Larval stage	Fused	?	Absent
		PATERSON, 1951	Adult	Fused	Separated	Present
Myobatrachidae	Heleophryne	VAN DER WESTHIZEN, 1961	Ontogeny	Fused	?	Absent
	Pseudophryne	JACOBSON, 1968	Larval stage	Fused	?	Absent
	Pleurodema	SOKOL, 1975	Larval stage	Fused	Separated	Absent
Ranidae	Rana	DE BEER, 1937	Ontogeny	Fused	?	Absent
		PUSEY, 1938	Ontogeny	Fused	?	Absent
		DE JONGH, 1968	Ontogeny	Fused	?	Absent
		PLASOTA, 1974	Ontogeny	Fused	?	Absent
Microhylidae	Breviceps	SWANEPOEL, 1971	Ontogeny	Fused	Fused	Absent
	Hypopachus	SOKOL, 1975	Larval stage	Fused	Fused	Absent

verify the state of the foramen for exit of trigeminal and facial nerves. The preparations studied are listed in app. 1. They have been deposited in the herpetological collections of the Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta, Argentina, and of the Fundación Miguel Lillo (FML), Argentina.

The whole mounts stained for peripheral nerves were prepared with Sudan Black B and maceration in trypsin (FILIPSKI & WILSON, 1984, 1985; NISHIKAWA, 1987). The 10 µm transverse serial sections were obtained using current histologic techniques, with hematoxylin-eosine coloration. The whole mounts stained for bone and cartilage were processed by the technique described in WASSERSUG (1976).

Observations and photographs were made using a stereomicroscope and an optical microscope.

We consider it necessary to explain that the term "root" is used for describing the preganglionic part of the nerve.

RESULTS

"LEPTODACTYLIDAE"

Ceratophrys cranwelli

The specimens at larval stage 31-34 show the trigeminal and facial nerves completely separated (fig. 1a-b) and the trigeminal ganglion is clearly defined. The skeletal preparations of larvae and adults present an undivided prootic foramen.

The facial nerve (VII) enters the rhombencephalon with the vestibulo-cochlear nerve (VIII) posterior to the root of the trigeminal nerve (fig. 2a). The facial root and the lateral-line nerves are together. They take a rostro-ventral course and diverge at the prootic foramen level in the prominent truncus hyomandibularis and the antero-dorsal lateral-line nerve. The truncus hyomandibularis lies below the ascendent process and subocular arch of palatoquadrate and extends toward the anterior elements of the hyobranchial skeleton (fig. 2b). The antero-dorsal lateral-line nerve is thin and branches in two rami.

The trigeminal nerve has its root in an anterior position and dorsal to the facial root (fig. 1a-b, 2b). The ganglion of this nerve is prominent (fig. 1b, 2b), and two branches diverge from it, namely: (1) the ramus ophthalmicus profundus lies ventral to the ascendent process of the palatoquadrate and runs anteriorly along the orbit wall toward the ethmoidal area (fig. 1a); (2) the truncus maxillo-mandibularis lies above the ascendent process of the palatoquadrate and diverges in the middle of the orbit into maxillaris and mandibularis branches (fig. 1a).

The specimen at stage 42 presents complete fusion of the trigeminal, facial, lateral-line, and vestibulo-cochlear roots. The roots and ganglia of trigeminal and facial nerves show no separation, and it is possible to differentiate the components of each nerve only at the prootic foramen level.
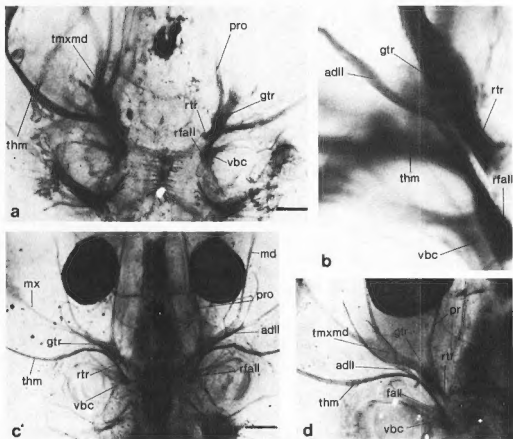
Fig. 1. – (a) Whole mount larval specimen of *Ceratophrys cranwelli* (stage 33) stained for peripheral nerves. The trigeminal, facial, lateral-line, and vestibulo-cochlear nerves are shown. Bar: 0.59 mm. – (b) Detail of trigeminal and facial nerves of the same specimen as seen from dorsal view. The trigeminal ganglion shows no connections with facial nerve. – (c) Whole mount larval specimen of *Lepidobatrachus llanensis* (stage 33) stained for peripheral nerves. Ventral view in which the trigeminal, facial, lateral-line, and vestibulo-cochlear nerves are observed. Bar: 1.20 mm. – (d) Detail of the trigeminal and facial nerves in same specimen as in (c). No fusion of trigeminal and facial nerves can be observed. – Abbreviations: adll, antero-dorsal lateral-line nerve; gtr, trigeminal ganglion; md, ramus mandibularis of trigeminus; mx, ramus maxillaris of trigeminus; pro, ramus ophthalmicus profundus of trigeminus; rfall, facial and lateral-line roots; rtr, trigeminal root; thm, truncus hyomandibularis; tmxmd, truncus maxillo-mandibularis of trigeminus; vbc, vestibulo-cochlear nerve.
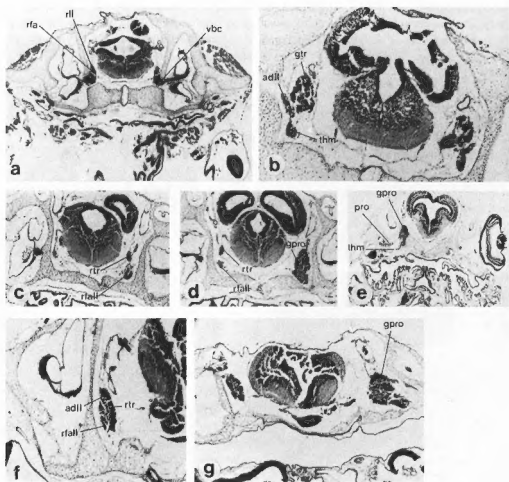
Fig. 2. — (a) Transverse section of larval specimen of *Ceratophrys cranwelli* (stage 33) at the level of the anterior half of the otic capsule; the facial and lateral-line roots are dorsal to the vestibulo-cochlear nerve. — (b) Transverse section of larval specimen of *Ceratophrys cranwelli* (stage 33) at the level of prootic foramen; trigeminal ganglion is defined and separated from the truncus hyomandibularis and antero-dorsal lateral line nerve. — (c) Transverse section of larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of anterior half of the otic capsules, in which the position of trigeminal, facial and lateral line roots is marked. — (d) Transverse section in larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of a plane anterior to that of (c). Structure of the prootic ganglion is shown. — (e) Transverse section of larval specimen of *Phyllomedusa sauvagii* (stage 33) at the level of the prootic foramen, in which the outlet of the ramus ophthalmicus profundus and truncus hyomandibularis of the prootic ganglion are observed. — (f) Tranverse section of larval specimen of *Dermatonotus muelleri* (stage 33) at the level of the otic capsule, in which the fusion of the trigeminal, lateral-line and facial roots is observed. — (g) Transverse section of larval specimen of *Dermatonotus muelleri* (stage 33) at the level of the anterior limit of the otic capsule, in which the prootic ganglion is observed. — Abbreviations: gpro, prootic ganglion; rfa, facial root; rll, lateral-line root; other abbreviations as in fig. 1.

Lepidobatrachus llanensis

The specimens at larval stages 31, 33 and 35 present complete separation of trigeminal and facial nerves (fig. 1c-d). The skeletal preparations of larvae and adults present a single prootic foramen.

The facial and lateral-line roots are in a posterior and ventral location with respect to the trigeminal nerve root (fig. 1d). The same trigeminal, lateral-line, and facial rami as those described for *C. cranwelli* are observed here (fig. 1c).

In the stage 37 specimen an approximation of the trigeminal and facial roots is observed. In the stage 42 specimen the ganglion of the trigeminal nerve and its root maintain their distinctness. The prootic ganglion is completely formed in the adult specimen.

Physalaemus biligonigerus

The specimens analyzed present trigeminal and facial roots separated from each other (fig. 3a), but anteriorly the prootic ganglion is already evident at the earliest stages (31-35). In whole mounts stained for bone and cartilage the prootic foramen is present. All the trigeminal, facial, and lateral-line rami described for *C. cranwelli* are recognized (fig. 3a). The separation of the nerve roots becomes less evident from stage 37 onward.

HYLIDAE

Hyla pulchella andina, Phyllomedusa sauvagii and *Scinax fuscovaria*

The ontogenetic sequences analyzed in these species present similar characteristics. The prefacial commissure is absent in larval and adult skeletal preparations. The formation of a prootic ganglion is evident (fig. 2d-e, 3b), although trigeminal and facial roots are separated (fig. 2c-d, 3b). In more advanced stages of development, the proximal portion of each nerve can only be recognized at the level of the roots.

MICROHYLIDAE

Dermatonotus muelleri

The prootic foramen is evident in osteologic preparations of larval and adult specimens. In the stage 27 specimen the truncus hyomandibularis and the trigeminal rami are clearly differentiated. They are independent; they enter the encephalon very close together. The lateral-line nerve crosses its fibers from the facial nerve to trigeminal nerve. No structure recognizable as a prootic ganglion can be distinguished. In stage 29, facial and trigeminal roots are fused. In the stage 33 specimen, facial and trigeminal nerves are integrated to form the prootic ganglion (fig. 2f-g, 3c-d).
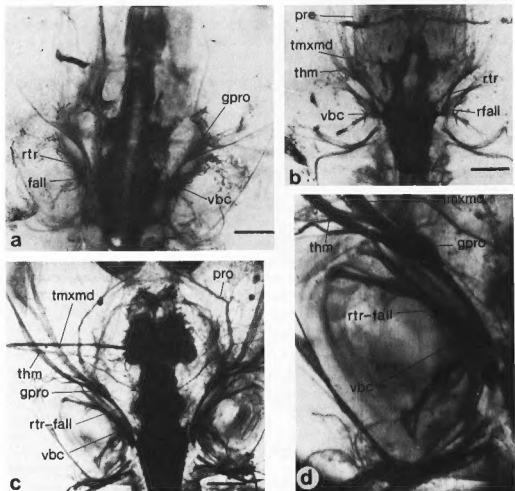
Fig. 3. — (a) Whole mount of larval specimen of *Physalaemus biligonigerus* (stage 33) stained for
peripheral nerves. The trigeminal, facial and vestibulo-cochlear nerves are indicated; the
trigeminal and facial-lateral-line roots are separated, but the presence of the prootic ganglion
is evident. Bar: 0.48 mm. — (b) Whole mount of larval specimen of *Phyllomedusa sauvagii*
(stage 33) obtained for peripheral nerves. The relations of trigeminal and facial nerves are as
in (a). Bar: 1 mm. — (c) Whole mount of larval specimen of *Dermatonotus muelleri* (stage 33)
obtained for peripheral nerves. The trigeminal, facial-lateral-line, and vestibulo-cochlear nerves
are illustrated. The trigeminal and facial-lateral-line roots are fused and the presence of the
prootic ganglion is evident. Bar: 0.52 mm. — (d) Detail of the trigeminal and facial
relationships in the same specimen as in (c). — Abbreviations: rtr-fall, trigeminal, facial and
lateral-line roots; other abbreviations as in fig. 1-2.

DISCUSSION AND CONCLUSIONS

The fusion of the trigeminal and facial ganglia presented by the anurans is an important character in their phylogeny, although, as mentioned, it has not been sufficiently investigated.

Some data from the literature are not entirely in agreement with observations made by SOKOL (1975). For instance, fusion of the trigeminal and facial ganglia has been described in postmetamorphic stages of *Discoglossus* (PUSEY, 1943; VAN EEDEN, 1951) — although trigeminal and facial ganglia can be clearly distinguished in *D. pictus* tadpoles (SCHLOSSER & ROTH, 1995) — and the presence of the prefacial commissure has been observed in *Hymenochirus* adults (PATERSON, 1951).

Information available for Pipanura is limited to seven genera of Mesobatrachia and only six genera of Neobatrachia. This information is insufficient for a discussion in depth, because for some taxa the character is described in a single larval stage, whereas for others the condition for adult forms or final stages of larval development has been superficially mentioned (see tab. 1).

The presence of a prootic ganglion and separate trigeminal and facial roots observed in *Physalaemus biligonigerus, Hyla pulchella andina, Phyllomedusa sauvagii* and *Scinax fuscovaria* larvae is in agreement with the observations in *Pleurodema* (SOKOL, 1975) and *Rana* (PUSEY, 1938). These species have the roots fused only near the ganglion. In later ontogenetic stages, this fusion extends proximally.

The trigeminal and facial nerves are found completely separated in larvae of *Ceratophrys cranwelli* and *Lepidobatrachus llanensis*, a characteristic which continues in *L. llanensis* until the start of metamorphosis. As this condition has not been referred to in Pipanura (tab. 1), the observations made on these species give grounds for not accepting the generalization proposed by SOKOL (1975) that Type IV, II and I larvae have a single prootic foramen and fused facial and trigeminal ganglia.

The early fusion of the trigeminal and facial roots and subsequent formation of the prootic ganglion observed in *Dermatonotus muelleri* larval development are in agreement with the information available on *Breviceps adspersus* (SWANEPOEL, 1971). Although ontogenetic aspects of the trigeminal and facial relations have not been described for *B. adspersus*, it is possible to deduce the process of fusion from roots to ganglia from the schema included (SWANEPOEL, 1971). In *Hypopachus* — another microhylid — a larval stage occurs in which ganglia and roots are found completely fused (SOKOL, 1975).

Although the information analyzed in this work is still very limited, the following conclusions are obtained:

(1) The prootic ganglion is not present in all neobatrachian larvae.

(2) In neobatrachian ontogeny, formation of the prootic ganglion can occur in two ways: in some species, formation of the ganglion precedes fusion of the trigeminal and facial roots; in others, fusion of the roots occurs first.

(3) There are interspecific heterochronies in the formation of the prootic ganglion.

(4) Analyses of the intracranial relation of the trigeminal and facial nerves during neobatrachian ontogeny will provide new information to clarify their phylogenetic relationships.

LITERATURE CITED

DE BEER, G. R., 1937. − The development of the vertebrate skull Oxford, Clarendon Press; reprint 1985, Chicago, Univ. Chicago Press. i-xlvi + 1-554, pl 143.

DE JONGH, H. J., 1968 − Functional morphology of the jaws apparatus of larval and metamorphosing Rana temporaria L. Neth. J. Zool., 18: 1-103.

DUELLMAN, W. E. & TRUEB, L., 1985. − Biology of amphibians. New York, McGraw-Hill, "1986"; reprint 1994, Johns Hopkins University Press: i-xxi + 1-670.

FILIPSKI, G. T. & WILSON, M. H. V., 1984. − Sudan Black B as a nerve stain for whole cleared fishes. Copeia, 1984: 204-208.

----- 1985. − Staining nerves in whole cleared amphibians and reptiles using Sudan Black B. Copeia, 1985: 500-502.

FORD, L. S. & CANNATELLA, D C., 1993. − The major clades of frogs Herp. Mon , 7: 94-117.

GOODRICH, E. S., 1930. − Studies on the structure and development of vertebrates. Chicago, Univ. Chicago Press; reprint 1958, Dover Publications Inc.: i-lxix + 1-837.

GOSNER, K. L., 1960 − A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica, 16: 183-190.

JACOBSON, C M., 1968. − The development of the chondrocranium in two species of the Australian anuran genus Pseudophryne Fitzinger. Aust. J. Zool., 16: 1-16.

LAURENT, R. F, 1986. − Sous-classe des Lissamphibiens (Lissamphibia) Systématique. In: P.-P. GRASSÉ & M. DELSOL (eds.), Traité de zoologie, tome 14, Batraciens, fasc. 1 B, Paris, Masson. 594-797.

NISHIKAWA, K. C., 1987. − Staining amphibian peripheral nerves with Sudan Black B. progressive vs. regressive methods. Copeia, 1987: 489-491.

ORTON, J. L., 1953. − The systematics of vertebrate larvae. Syst. Zool., 2: 63-75.

PATERSON, N. F., 1945. − The skull of Hymenochirus curtipes. Proc. zool. Soc. London, 115 327-354.

PLASOTA, K., 1974 − The development of the chondrocranium (neurocranium, and the mandibular and hyoid arches) in Rana temporaria L. and Pelobates fuscus (Laur.). Zool. Pol., 24 99-118.

PUSEY, H K , 1938. − Structural changes in the anuran mandibular arch during metamorphosis, with reference to Rana temporaria. Q. J. micr. Sci., 80 479-465.

----- 1943 − On the head of the leiopelmid frog, Ascaphus truei. I The chondrocranium, jaws, arches, and muscles of a partly-grown larva. Q. J. micr. Sci., 84. 105-185.

ROČEK, Z , 1981. − Cranial anatomy of frogs of the family Pelobatidae Stannius, 1856, with outlines of their phylogeny and systematics. Acta Univ. Carolinae, Biol.: 1-164

SCHLOSSER, G. & ROTH, G., 1995. − Distribution of cranial and rostral spinal nerves in tadpoles of the frog Discoglossus pictus (Discoglossidae). J Morph , 226: 189-212

SOKOL, O. M., 1975. The phylogeny of anuran larvae: a new look. Copeia, 1975: 1-23.

----- 1977 The free-swimming Pipa larvae, with a review of pipid larvae and pipid phylogeny (Anura: Pipidae). J. Morph., 154: 357-425.

----- 1981. – The larval chondrocranium of *Pelodytes punctatus*, with a review of tadpoles chondrocrania. *J. Morph.*, **169**: 161-183.

STARRETT, P. H., 1973. – Evolutionary patterns in larval morphology *In:* J L VIAL (ed.), *Evolutionary biology of the anurans*, Columbia, Univ. Missouri Press: 252-271.

STEPHENSON, N. G., 1950 – On the development of the chondrocranium and visceral arches of *Leiopelma archeyi. Trans. zool. Soc. London*, **27**: 203-253.

SWANEPOEL, J. H., 1970. – The ontogenesis of the chondrocranium and of the nasal sac of the microhylid frog *Breviceps adspersus pentheri* Werner. *Ann. Univ. Stell.*, **45** (A): 1-119.

VAN EEDEN, J A., 1951. – The development of the chondrocranium of *Ascaphus truei* Stejneger, with special reference to the relations of the palatoquadrate to the neurocranium. *Acta zool*, **32**: 1-136.

WASSERSUG, R. J., 1976. – A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol.*, **51**: 131-134.

WESTHUIZEN, C M. V. D., 1961. – The development of the chondrocranium of *Heleophryne purcelli* Sclater with special reference to the palatoquadrate and the sound-conducting apparatus. *Acta zool.*, **42**: 1-72.

Corresponding editor: Marvalee H. WAKE.

APPENDIX 1
LIST OF SPECIMENS EXAMINED

FAMILY LEPTODACTYLIDAE

Ceratophrys cranwelli. – MCN 021: whole mounts stained for peripheral nerves of 20 specimens at stages 31-34 and 2 specimens at stage 42; transverse serial sections of 2 specimens at stage 33; whole mounts stained for bone and cartilage of 10 specimens at stages 31-34. FML 4534: whole mounts stained for bone and cartilage of 7 specimens at stages 40-46. FML 4573: dry skeleton of 1 adult specimen. FML 4574: dry skeleton of 1 adult specimen.

Lepidobatrachus llanensis. – FML 4678: whole mounts stained for peripheral nerves of 5 specimens at stages 31, 33, 35, 37 and 42; whole mounts stained for bone and cartilage of 4 specimens at stages 31, 33, 37 and 44. FML 0420: dry skeleton of 1 adult specimen. FML 1016: dry skeleton of 1 adult specimen. FML 5220: dry skeleton of 1 adult specimen. FML 5221: dry skeleton of 1 adult specimen. MCN 081: dissection of 1 adult specimen.

Physalaemus biligonigerus. – MCN 043: whole mounts stained for peripheral nerves of 22 specimens at stages 31-42; whole mounts stained for bone and cartilage of 10 specimens at stages 32-41. MCN 157: whole mounts stained for bone and cartilage of 4 adult specimens.

FAMILY HYLIDAE

Hyla pulchella andina. – MCN 024: whole mounts stained for peripheral nerves of 14 specimens at stages 31-42; whole mounts stained for bone and cartilage of 10 specimens at stages 33-39. MCN s/n: whole mounts stained for bone and cartilage of 2 adult specimens.

Phyllomedusa sauvagii — MCN 061: whole mounts stained for peripheral nerves of 18 specimens at stages 31-42; transverse serial sections of 2 specimens at stage 33; whole mounts stained for bone and cartilage of 7 specimens at stages 33-38. FML 3823: whole mounts stained for bone and cartilage of 2 adult specimens.

Scinax fuscovaria. — MCN 027: whole mounts stained for peripheral nerves of 19 specimens at stages 31-41; whole mounts stained for bone and cartilage of 7 specimens at stages 33-38. MCN 072: whole mounts stained for bone and cartilage of 2 adult specimens.

FAMILY MICROHYLIDAE

Dermatonotus muelleri. — FML 4694: whole mounts stained for peripheral nerves of 6 specimens at stages 27, 29, 30, 33, 35 and 37; whole mounts stained for bone and cartilage of 3 specimens at stages 33-35. MCN 056: whole mounts stained for peripheral nerves of 5 specimens at stages 31-36; transverse serial sections of 3 specimens at stage 33; whole mounts stained for bone and cartilage of 7 specimens at stages 35-41 FML 1074: whole mounts stained for bone and cartilage of 1 adult specimen.